THE FATE OF THE UNFERTILIZED EGG IN THE WHITE MOUSE

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It has long been known that in mammals many more eggs are ovulated than develop into embryos, due in part to the failure of the spermatozoa to reach them, and perhaps more often to the inability of the eggs to implant themselves in the uterus. Such eggs are said to degenerate, but the story of their fate has been recorded in only a few instances. Since ovulation in the mouse is independent of copulation, this animal was selected for the investigation. It is natural to suppose that the process would be similar to the degeneration of the ovarian egg which has been prevented from leaving the ovary, the process termed follicular atresia.

The atresia of ovarian eggs in mammals has been described in detail by many investigators, but the degeneration of the unfertilized egg in the Fallopian tube and uterus has been given but little special study. Heape ('05) working on the domestic rabbit, mentions the finding of eggs in the tube which for some reason or other had not been fertilized and were in a process of degeneration. Hartman ('16), in his study of the early development of the American opossum, also refers to the great number of unfertilized eggs, approximately 50 per cent., which he found in the uterus, in an earlier or later stage of degeneration. He ventures the opinion that these eggs would eventually leave the uterus.

Students of atresia have usually recognized that there is an early disturbance of the nucleo-cytoplasmic relationship, although they have not always given it that name, and that it was followed by a rearrangement of the cytoplasm. Some writers however insist that a true parthenogenetic cleavage may take place, and continue in a more or less normal manner up to, in some cases,

the ten or twelve cell stage before the degenerative processes get the upper hand.

The purpose of the present investigation is to describe step by step the degeneration, whether it be fragmentation or parthenogenetic development, of the unfertilized egg in the white mouse, and to compare the process with follicular atresia in the mouse and other mammals.

The work was undertaken at the suggestion of Prof. W. R. Coe, and done under his supervision. I am pleased to acknowledge his many valuable suggestions during the course of the investigation.

LITERATURE.

Kingery ('14) describes the method of atresia in the follicles of young mice. In his opinion the process is one of degenerative fragmentation only, that the spindle fibers of the second maturation spindle break, forming aster-like radiations and that later the achromatic fibers entirely disappear. Then the loose chromosomes form vesicles, and occasionally these fuse together to form larger vesicles. This disturbs the nucleo-cytoplasmic relationship, and an attempt to counter balance is made by the cytoplasm breaking up and surrounding the nuclei, the larger amount of cytoplasm enclosing the larger nuclear vesicles. In some cases the cytoplasmic fragments have no nucleus whatever. These fragments with or without nuclei are later absorbed, probably by the action of phagocytic cells of follicular origin.

Newman ('13) found that in the armadillo the first maturation spindle was formed in the ovary and that the egg then waited for ovulation. Eggs not located near the periphery of the ovary were not ovulated. In such cases Newman found that only a small percentage cut off the first polar body and that only three per cent. in several hundred cases gave off the second polar body, while ninety per cent. of the eggs were struck by a process of follicular atresia, which is either a cytolysis or a parthenogenetic development. He considers only the latter contingency. In such eggs there is first a casting out of the deutoplasmic mass from the formative cytoplasm. This he considers to be an act of rejuvenation on the part of the dying cell. Released from the burden of the yolk the cell is better

able to carry on the natural processes. The abstrictions of the yolk often look like multicellular structures, but Newman thinks this appearance is due to fixation. Some of these yolk fragments which he calls cytoids contain deutoplasmic granules.

Newman finds numerous tri- and multipolar spindles. These, he thinks, would result in nests of nuclei without division of the cytoplasm. Sometimes instead of a single resting nucleus formed from the maturation spindle, two nuclei might be formed instead, without the extrusion of the polar body, and suggests that from these double nuclei, the multipolar spindles might be formed. As the number of chromosomes is more than the haploid number such spindles cannot be maturation spindles. In those cells with a true cleavage spindle, Newman is inclined to the view that no polar bodies have been given off. The process is in his opinion a true parthenogenesis, but development does not proceed beyond the eight cell stage and even at that time advancing degenerative processes are to be seen.

Heape ('05) found that in the domestic rabbit copulation acted as a stimulus to certain internal rearrangements which ended in severing the ovum from its source of nourishment. At about nine hours after copulation the two polar bodies are formed and ovulation takes place about an hour later. He says: "Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilized; if from any cause fertilization is not effected, the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are from time to time to be seen in the Fallopian tubes." It is therefore necessary for spermatozoa to be at the top of the Fallopian tube because, unlike the condition in the mouse, the ovum is dehisced from the ovary without any discus cells to provide it with food.

Bonnet ('00) reviews the different theories of degeneration and takes the view that the various spindles figured by the exponents of the parthenogenetic idea are to be considered more or less abnormal maturation spindles, and not cleavage spindles.

Van der Stricht ('01), in his study of follicular atresia in the bat, comes to the conclusion that the oöcyte of the second order forms a cleavage spindle and divides parthenogenetically. He

has traced an apparent normal cleavage as far as the six-cell stage. He also described multipolar spindles and occasionally two spindles in the same egg. The first polar body is occasionally seen to divide.

Kirkham ('07) gives an excellent review of the literature on the early development of mammalian eggs previous to 1907. The common occurrence of spindles in the polar body led him to conclude that the polar body would divide mitotically. Abnormal eggs containing tripolar spindles and in one case an egg containing two spindles were observed. Kirkham agrees with Sobotta ('95) and Rubaschkin ('06) that an egg never develops after the formation of the first polar body and the second polar spindle unless fertilization takes place, the egg degenerating within the ovary or in the Fallopian tube.

Athias ('09) also takes the view that the process is entirely one of degenerative fragmentation.

Material and Methods.

The mouse is an animal well adapted to such a study, for we know from the investigations of Sobotta, Kirkham, and later of Mark and Long, that ovulation takes place without the necessity of copulation in from thirteen and three-quarter to twenty-eight and one-half hours (Mark and Long) after parturition. The sexes were kept together in suitable cages and the females examined frequently for signs of pregnancy. Whenever a female was found to be pregnant she was placed in a cage and examined each morning. As soon as a litter was found it was removed, and a record of the time made. It is from this time record that all the ages for the various eggs are figured.

Animals were killed at intervals varying from one and one half days to four days and nine hours after the finding of the litter. The body was immediately cut open and the ovaries with tube and uterus removed and placed in the fixing solution. For this purpose Zenker with acetic, and Carnoy's 6–3–1 solution, were found to be about equally good. For tube eggs strong Flemming proved excellent. The prepared sections were stained in Heidenhain's iron hæmatoxylin, Ehrlich's hæmatoxylin, or Flemming's Triple stain, usually with suitable counter stain.

Observations.

Thirty-six hours after finding the litter, eggs are almost certain to be found in the upper part of the Fallopian tube. They always show the second maturation spindle and may or may not have the polar body attached. If present, the polar body has the chromosomes arranged in the form of a spindle (Fig. 1.) During the next twelve hours there is a change in the polar bodies. The chromatin of the spindle breaks down and arranges itself into one, two or many resting nuclei. The formation of two nuclei is well illustrated in Fig. 2. Here the vesicles have been formed but a number of chromosomes or chromatin bodies have not yet entirely lost their individuality. To one side of the polar body a small body, the first indication of a mitochondrial substance, may be seen. Fig. 3 shows a later stage in which the chromatin of the polar body has formed eight vesicle-like nuclei. Since eggs with three or more polar bodies are not infrequent (see Fig. 4), one is led to believe that the cytoplasm segments, forming itself around the various vesicles. Long and Mark ('11) find that the first polar cell often divides amitotically. They consider that this aids the degeneration and absorbtion of the polar bodies due to the increase in the exposed surfaces. Kirkham also, in work which has never been published, has observed that the first polar cell occasionally divides forming a number of fragments.

The achromatic fibers of the second maturation spindle disappear early, since in only a few cases have they been noted in eggs as old as forty-eight hours. The spindle stands out clear and distinct with the chromosomes in their natural relations but no signs of fibers can be observed.

Between forty-eight and seventy-two hours a renewal of activity begins in the unfertilized egg. Its effect is first apparent in the second maturation spindle, which may break down and form a single resting nucleus. Three eggs from a seventy-six hour mouse all have a single nucleus both in the eggs and in their polar bodies. More often however numerous vesicles instead of one result from the breaking down of the spindle. An examination of Fig. 5 will make it clear how this takes place. This egg is from a mouse in which one of the uterine horns was

closed by a tumor, thus preventing access of the sperm to the eggs on that side. The mouse was killed sixty hours after finding the litter. In the tube on the occluded side, two eggs were found, one in the second maturation spindle stage, and the other showing the breaking down of the spindle. In the other horn fertilization had taken place and the eggs were in the four-cell stage. In the figure it will be noted that the chromatin of the spindle has for the most part gone into the formation of a circular vesicle, in which a number of chromosomes remain practically unaltered.

A later stage picturing the breaking down of the spindle is that of Fig. 6. Here the lobes are larger and have become separated from the central vesicle. All the chromatin is now found in the walls of the various vesicles, none remaining as chromosomes.

The loss of the achromatic fibers and the breaking down of the spindle is probably due, as suggested by Kingery ('13), to the degenerative changes appearing quite early in the cytoplasm. This is shown by the fact that the cytoplasm stains more deeply than in normal eggs. Numerous dark staining granules are seen, particularly near the periphery of the egg. These constitute the mitochondrial bodies.

Kingery ('13) is of the opinion that the spindle fibers of the second maturation spindle break and free the chromosomes, which then migrate into the cytoplasm and form numerous nuclear vesicles. In my material the achromatic fibers disappear early, and from Figs. 5 and 6 it would appear that the vesicles may be formed *in situ*, and not necessarily from chromosomes which have wandered from the place of the original spindle.

In Figs. 7, 8 and 9 a different process of spindle disintegration is seen. These three eggs are all from the same mouse and were located close together in the lowest portion of the tube very near the uterus. From Fig. 7, it is evident that while the spindle fibers have disappeared; the chromosomes, although contracted and hugged closely together, still retain the form of a second spindle. Several chromatic fragments have evidently broken off and are seen scattered in the cytoplasm. The succeeding stage may be noted in Fig. 8. The individual chromosomes

have separated from their spindle position and lie scattered in the cytoplasm. One of the chromosomes shows the beginning of vesicle formation, which is seen completed in Fig. 9. Fig. 11 shows essentially the same condition. The polar body has divided and several small phagocytes may be seen just inside the zona. In Fig. 10 the chromatin is arranged around the periphery of the vesicle and not clumped to one side as in Fig. 9. One notices quite a similarity between these vesicles and those in process of formation in Fig. 6, and suggests that they may have been preceeded by such a stage.

After the formation of these numerous nuclear vesicles, the fragmentation of the cytoplasm takes place. Figs. 16 and 17, adjacent sections of a seventy-two hour egg, indicate that the fragmentation may take the form of a protoplasmic budding. The large cell shown in the figure has constricted off two fragments and is forming others. Adjoining sections show five additional fragments, making seven in all. Nuclear material is present in only two of the seven fragments. Of course one cannot say that all such fragments are constricted off from the egg, for there is always the possibility that some of them may have come from the polar bodies. It has been shown by Hartman ('16) in the opossum, and by Newman for the armadillo that normally one of the first activities of the unfertilized egg is the unburdening itself of the deutoplasmic material. This is done without assistance of any spindle or apparent nuclear influence. While it cannot be said that the fragments budded off in the mouse are exclusively of yolk material, the process is essentially a similar one.

Figs. 13, 14 and 15 are later stages in the fragmentation process. They represent what Kingery ('13) calls the "morula" stage. Figs. 14 and 15 represent two adjoining sections of the same "morula," which consists of about twenty-one cytoplasmic fragments.

We have seen how the second maturation spindle breaks down and forms either a single resting nucleus or numerous nuclei, and have traced the latter to the "morula" stage. When, on the breaking down of the spindle the egg is able to form a single or only a few resting nuclei, it would seem to indicate exceptional

vitality and a close approximation of the normal condition. In such an egg the process of atresia would naturally be somewhat delayed. The egg with but one or two nuclei does not fragment immediately, and it is possible that such eggs may pass from the body, as suggested by Hartman ('16) for the unfertilized egg of the opossum. This possibility is well illustrated in Fig. 18, which shows an unfertilized egg one hundred and five hours after finding the litter. Only two nuclei are present. The mitochondrial bodies (*b*) can be distinguished from the nuclei by their peculiar crystalline appearance and elliptical shape. Although these bodies are found in most of the fragmented segments to be described later, they are smaller and stain less intensely in hæmatoxylin. They are surrounded by a light area which gives them the appearance of having shrunken from the cytoplasm. In Fig. 19 the mitochondrial bodies are seen to be smaller but more numerous. In the two upper fragments they did not cut cleanly but were pushed aside by the knife. This would seem to indicate that they were much more compact and dense than the surrounding cytoplasm.

Hartman ('16) finds a similar structure in the egg of the opossum, and describes it as a homogeneous, non-granular body staining pink or lavender in iron hæmatoxylin, and bordered by a light-colored band.

The multinucleated eggs soon break up into a number of fragments, often forming two or more parts very nearly of the same size and often appearing like normal cleavage stages, were it not for the presence of extra nuclei. Figs. 12, 13 and 14 show different stages in this process.

The ultimate fate of the fragmented egg will now be considered. Very early in the degenerative process, phagocytic cells are found inside the zona pellucida, between the cytoplasm and the zona. It is impossible to mistake these cells for fragments of either the egg or polar bodies for they appear almost like transparent vesicles with the deep staining nuclear material either scattered as granules or irregularly clumped in a way which immediately suggests their origin as polymorphonuclear leucocytes. Kingery described similar cells but thought them of follicular origin. The number of these phagocytic cells inside the zona varies,

eight or ten being the largest number found. These cells probably aid in the disintegration of the periphery of the cell. This process may begin some time before fragmentation sets in, as is evidenced by a study of Fig. 9. Here the space at one end of the egg filled with dark granules indicates that the cytolysis of the cytoplasm has begun.

The zona persists in nearly all cases as far as the eggs have been followed. Fig. 6 shows one of the exceptions, for here the zona is gone and many dark staining fragments which may have come either from the polar body (which is now very small) or from the broken down zona, are seen surrounding the egg. Several phagocytic cells, one of which is figured, are to be seen among the fragments. Although the zona is present in almost all cases, a careful examination usually brings to light several breaks, and it is probably through these that the phagocytic cells are able to reach the egg.

Figs. 19 and 20 show the last stages in the disintegration and absorbtion of the fragments by the phagocytes. Some of the fragments appear as mere shells with only occasional granules, while the outer edge of others shows a honeycombed condition due to excessive vacuolization.

It is found that the unfertilized egg completes its passage through the Fallopian tube and enters the uterus about the end of the third day or almost at exactly the same time as given by Sobotta for the normal segmenting egg.

Smith ('17), basing ovulation as occurring twenty hours after parturition, finds that unfertilized eggs of the mouse enter the uterus about 76 hours after ovulation and are found in the last fold of the uterus at 88 hours.

The final dissolution of the fragmented egg evidently is completed early on the fourth day, since out of eleven mice older than four days, all showing corpora lutea in the ovaries, indicating that ovulation had taken place, in only three could eggs be found. A single egg was found in the uterus of a mouse one hundred and five hours after parturition (Fig. 18).

In not a single case has anything approaching a cleavage spindle as described in atresic ova by Spuler, Loeb or Newman, been observed, but it is not to be interpreted that they may

not occur, because cleavage spindles are so short lived that the study of a much greater number of eggs would be necessary before coming to any such conclusion.

SUMMARY.

The cytological changes which the unfertilized egg of the mouse undergoes in the Fallopian tube and uterus are closely similar to those which occur in the eggs of atresic follicles in the ovary.

The processes are considered degenerative and have only a superficial resemblance to parthenogenetic development.

The breaking down of the second maturation spindle of the unfertilized egg usually results in the formation of several or many nuclei. Rarely a single nucleus is formed, in which case the egg does not fragment rapidly and may pass from the uterus before the degenerative processes are complete.

The eggs with many nuclei divide into numerous cells, of which some are provided with degenerating nuclei. These are further disintegrated and absorbed by phagocytic cells, which make their way into the egg probably through breaks in the zona. The phagocytes from their appearance are polymorphonuclear leucocytes, and they evidently act on the cytoplasm causing a vacuolization of its outer portions and a later crumbling of its periphery; the end being a complete disintegration of the egg and its absorption by the phagocytes.

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EXPLANATION OF PLATES.

All the drawings are from sections of unfertilized eggs in the Fallopian tube or uterus. They were made with the aid of a camera lucida, and with the exception of Fig. 5 are reproduced at a magnification of 900 diameters.

ABBREVIATIONS.

a, zona. *b*, mitochondrial body. *c*, phagocytes. *d*, vacuole. *e*, degenerating protoplasm.

PLATE I.

FIG. 1. (48 hours.) Egg with the second maturation spindle. Polar body shows chromosomes arranged on the spindle fibers. Eggs of this type, showing no changes are found in the tube up to forty-eight hours after parturition.

FIG. 2. (56 hours.) In this egg the spindle is present but the chromosomes seem to have clumped together more than in Fig. 1. The spindle of the polar body has disappeared and in its place are seen two resting nuclei.

FIG. 3. Polar body (60 hours) from same mouse as Fig. 5 showing many resting nuclei formed from the breaking down of the polar spindle or by the chromatin fragments often observed in the polar body.

FIG. 4. (48 hours.) Showing the fragmentation of the polar body. Here the first polar body has divided once and one of the parts has redivided. The spindle cut somewhat obliquely is one of few showing good achromatic fibers. These fibers seem to end in a centrosome-like body.

FIG. 5. (60 hours.) Chromosomes of the second maturation spindle breaking down and forming resting nuclei. Vesicular lobes are seen extending out from the main vesicle. Quite a number of the chromosomes have not yet entered into the process and are seen in the lobes of the forming nuclei. This mouse had a tumor in one of the uteri which prevented fertilization, while on the other side, eggs in the tube had reached the four cell stage. (× 1,800.)

FIG. 6. (78 hours.) A later stage of vesicle formation from the maturation spindle. Chromatin all in the vesicles, no chromosomes as such. This was one of the few eggs in which the zona was missing. Many dark staining granules surrounded the egg and in among them several phagocytes. One phagocyte shown at left of polar body, which is much smaller than normal, suggesting that the phagocytes have been acting upon it and probably upon the zona also.

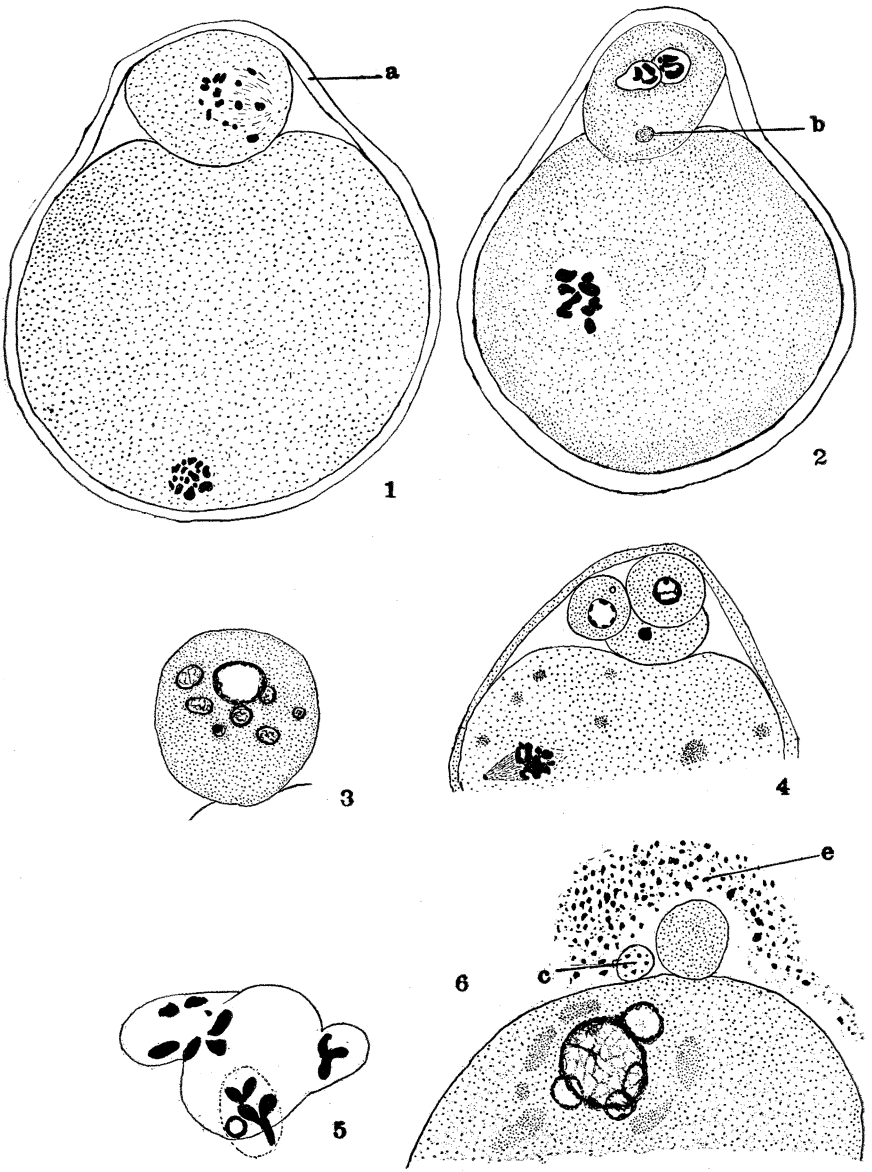

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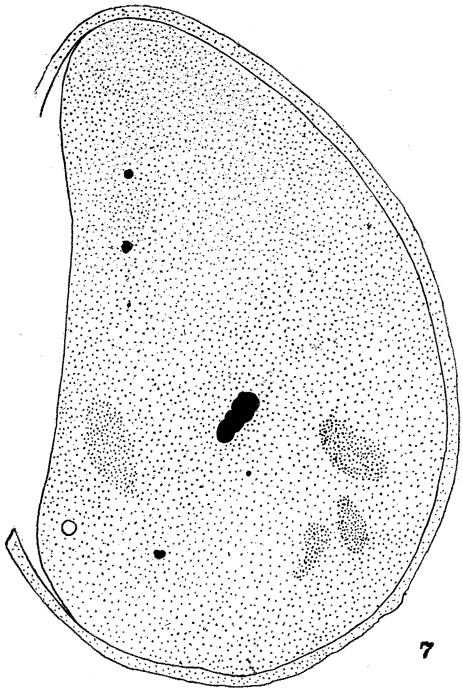
PLATE II.

FIG. 7. (72 hours.) Spindle fibers missing, chromosomes of spindle hugged closely together. Several parts separated and out in the cytoplasm.

FIG. 8. (72 hours.) A later stage of the above. Chromosomes scattered in the cytoplasm. One showing the beginning of vesicle formation. Several phagocytes are present inside the zona. Cytoplasm near open end shows beginning of disintegration.

FIG. 9. (72 hours.) Completion of vesicle formation begun in Fig. 8. The chromatic material clumped to one side of the vesicle. Quite a few small particles of disintegrated cytoplasm may be seen inside the zona indicating the activity of the phagocytes.

FIG. 10. (72 hours.) Reconstruction showing ring-like formation of nuclear material. Possibly a succeeding stage to Fig. 6. A phagocyte cell is noticed just outside the zona, which is very faintly stained.



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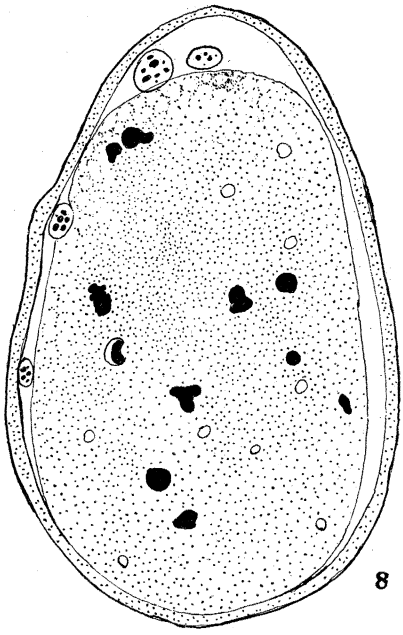

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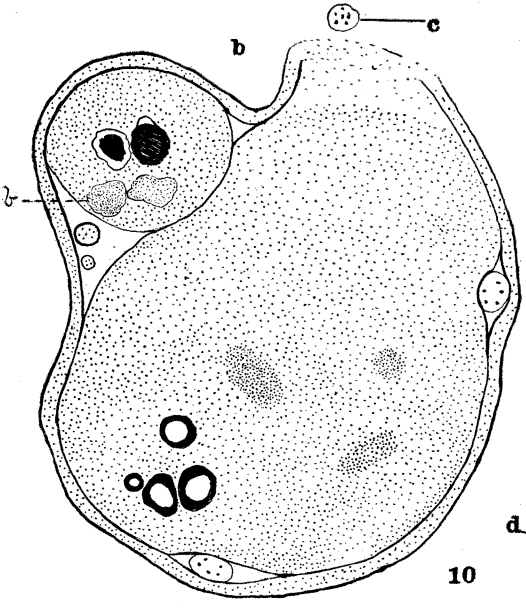

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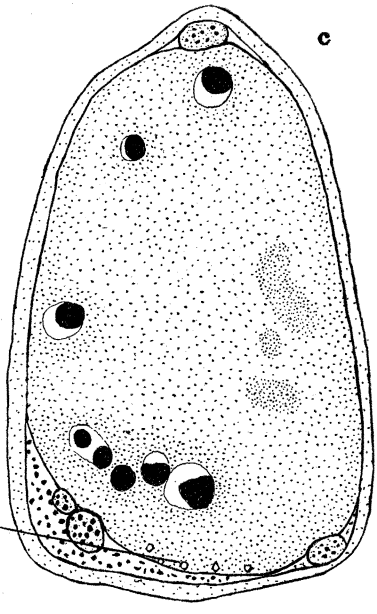

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PLATE III.

FIG. 11. (72 hours.) Egg showing four resting nuclei, and a number of mitochondrial bodies. In addition a divided polar body and two or more smaller structures which are probably phagocytic cells.

FIG. 12. (72 hours.) Fragmented egg looking very much like a normal two-cell stage, except that each blastomere has two or three nuclei. The nuclei contain nucleoli and appear perfectly normal.

FIG. 13. (81 hours.) A later stage of fragmentation. A large resting nucleus is seen in one of the larger segments, while other fragments have none. In two of the cells the mitochondrial body is quite conspicuous.

FIG. 14. Egg of uncertain age, consisting of twenty-one fragments. Numerous phagocytic cells are seen in and about the fragments. Zona entirely missing.

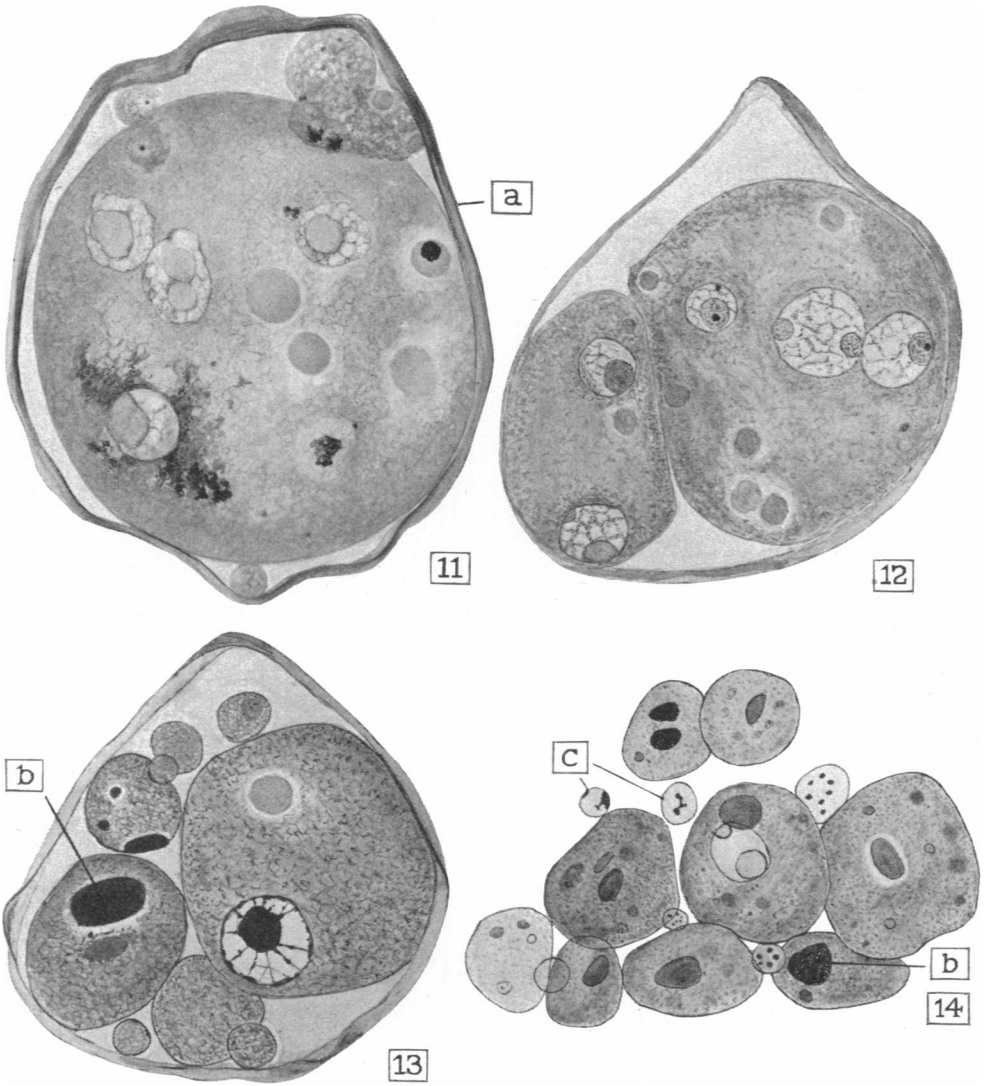

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PLATE IV.

FIG. 15. Drawing of adjacent section to that shown in Fig. 14.

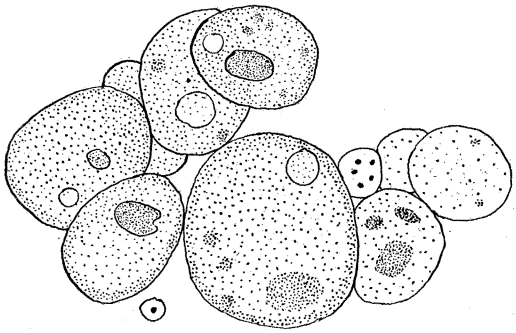
FIG. 16. (72 hours.) Egg showing the formation of protoplasmic fragments independent of any spindle. These are later pinched off and form small segments inside the zona.

FIG. 17. Drawing of adjacent section to that shown in Fig. 16. A study of all the sections of this egg shows that seven fragments have been pinched off, and that of these only two appear to contain nuclear material.

FIG. 18. (105 hours.) Egg is without zona, contains two nuclei and two immensely large refractive mitochondrial bodies. The polar body still remains attached to the egg.

FIG. 19. An 81-hour egg showing phagocytic effect. In two upper cells the mitochondrial bodies have been pulled out of the cytoplasm towards the left by the knife, leaving clear spaces where they originally were, indicating the solid character of these structures. Many vacuoles are seen around the border of the fragments. Between the cells dark stained particles of degenerated cytoplasm is being absorbed by the phagocytes.

FIG. 20. (81 hours.) A later stage than Fig. 19. In one of the fragments hardly any content is to be seen except a few scattered granules.



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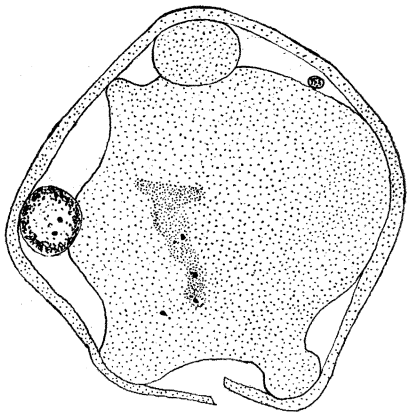

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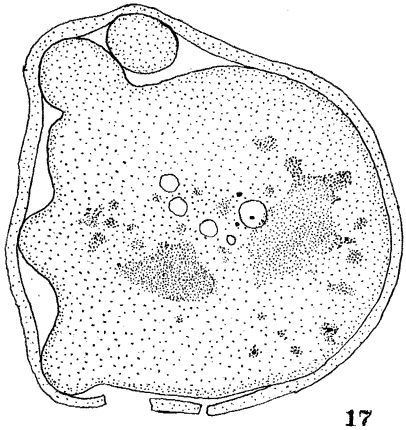

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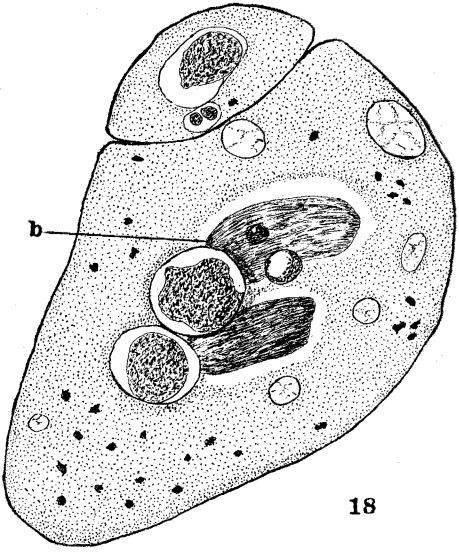

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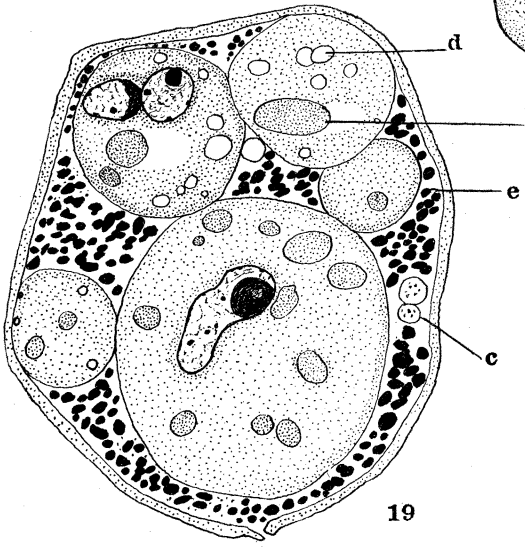

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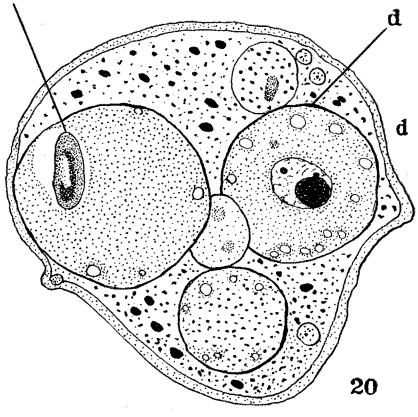

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